Synthesis, characterization, spectrophotometric and electrochemistry studies, and DNA cleavage of copper(II) complexes of a new azacrown bis-macrocycle and its mono-cyclic analogue

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Abstract: A new azacrown bis-macrocycle (5) and its mono–cyclic analogue (7) were synthesized and characterized by FT-IR, 1H NMR, 13C NMR, DEPT 13C NMR, MS, and elemental analysis. The reaction with copper(II) nitrate yielded the corresponding complexes, formulated as Cu(5)(NO3)2 · 3H2O (8), and Cu(7)(NO3)2 · 2.5H2O (9). Also the stoichiometries of the complexes were determined in alcoholic solution and the results show that for both complexes the ratio of ligand to metal was 1:1 in methanol. The redox behavior of both complexes has been studied by cyclic voltammetry in DMF. Cyclic voltamogram of 8 shows quasi-reversible CuII/CuI redox couple whereas 9 shows a reversible CuII/CuI redox couple. Mono- and bis-macrocycle copper(II) complexes (8 and 9 respectively) cleaved plasmid pGS2 DNA by using an oxidative mechanism with 3- mercaptopropionic acid (MPA) as the reductant under aerobic conditions. The bis-macrocycle copper(II) complex 8 showed higher cleavage efficiency than their mono-macrocycle analogue 9 at the same Cu2+ concentration.

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Synthesis, characterization, spectrophotometric and electrochemistry studies, and DNA cleavage of copper(II) complexes of a new azacrown bis-macrocycle and its mono-cyclic analogue

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Abstract

A new azacrown bis-macrocycle (**5**) and its mono–cyclic analogue (**7**) were synthesized and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT ^{13}C NMR, MS, and elemental analysis. The reaction with copper(II) nitrate yielded the corresponding complexes, formulated as Cu(**5**)$(NO_3)_2.3H_2O$ (**8**), and Cu(**7**)$(NO_3)_2.2.5H_2O$ (**9**). Also the stochiometries of the complexes were determined in alcoholic solution and the results show that for both complexes the ratio of ligand to metal was 1:1 in methanol. The redox behavior of both complexes has been studied by cyclic voltammetry in DMF. Cyclic voltamogram of **8** shows quasi-reversible Cu^{II}/Cu^{I} redox couple whereas **9** shows a reversible Cu^{II}/Cu^{I} redox couple. Copper(II) complex **8** has a good activity to degrade supercoiled plasmid pGS2 DNA by using an oxidative mechanism to nick and linear forms under aerobic conditions with 3-mercaptopropionic acid (MPA) as a reductant.

Keywords

Linked Macrocycle; Azacrown; Copper(II); DNA Cleavage; Cyclic Voltammetry

Introduction

Recently there has been increasing interest in the design and synthesis of larger molecular units, incorporating multi-metal ion binding sites [1, 2]. Macrocyclic rings because of their often unique properties have long been generated by the prospect that such systems may serve as models for charge transfer, electron transport and allosteric behavior often observed in biochemical systems [3 - 5]. Macrocycles are desirable moieties for generating multicomponent molecular systems since they often give rise to kinetically and thermodynamically stable complexes [6]. One category of macrocyclic systems is composed of linked ring ligands which are capable of binding simultaneously to metal ions [7]. While there are now a considerable number of linked macrocyclic systems incorporating two macrocyclic rings [8 – 12] systems incorporating three or more rings are considerably less common. Several research groups have reported linearly-linked tris-macrocyclic species based on aza crowns [13 – 21]. The success of the above studies has rested upon the application of the N-protecting groups, Boc as a flexible protecting group to the step-wise synthesis of the linked-ligand species [22, 23]. A wide variety of linked macrocyclic ligands, including species covalently linked by secondary amines, has been reported. [9, 10, 24, 25]. In this context, we have synthesized a new bis macrocycle (**5**) with their two rings linked by a para xylene moiety and its mono marcocyclic analogue **7** (Scheme 1) and have studied their behavior towards the complexation of Cu(II). We also describe here electrochemistry of the obtained complexes **8** and **9** in solution and DNA cleavage by complex **8**.

Experimental Section

General procedures

All chemicals were from Aldrich, Merck and Fluka and used without further purification. Some organic reactions were performed under N_2 and reactions were monitored by TLC on Merck silica gel and aluminum plates. Column chromatography was carried out with Merck silica gel (> 230 mesh) and Aldrich neutral aluminum oxide (100-300 mesh). NMR spectra were recorded on a Bruker AV2 (400 MHz) and AV1 (500 MHz) spectrometer. Chemical shifts are relative to residual solvent protons or TMS as references. UV-Vis absorption spectra were recorded by using UV-Vis (Cary 50, Version No 1.00) spectrophotometer. Infrared spectra were measured with a Perkin Elmer FT-IR spectrophotometer. Electrospray ionization spectra (ESI-MS) and Matrix–assisted laser desorption/ionozation (MALDI) were obtained on a Esquire HCT Spectrometer from Bruker (Bremen, Germany) and (MSOCI_MALDI, Matrix: HCCA, solvent: acetonitrile: water 1:2, 0.1 % TFA, Laser at (365 nm)) in the Institute of Inorganic

Chemistry at the University of Zurich. Elemental analyses were performed on a Leco CHNS-932 elemental analyzer. CV measurements were performed using a glassy carbon as working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl reference electrode. The ferrocene/ferrocenium couple (Fc/Fc^+; $E_{1/2}$ = 505 mV) was used as a standard but all potentials in the paper are referenced to the Ag/AgCl reference electrode. Solutions containing 10^{-3} M complexes and 0.05 M KNO_3 as supporting electrolyte were deoxygenated by a stream of high purity nitrogen for at least 10 min.

Starting Materials

2-{3-(2-formyl phenoxy)-2-hydroxy} benzaldehyde **1** was synthesized as described in our works [26, 27] and macrocycle **2** was synthesized and purified as described previously [28]. The reaction of **2** with di-tert-butyl dicarbonate $(Boc)_2O$ in methanol produced the N-protected macrocycle **3** in good yield 71 % [29], Scheme 1.

White crystals of **1** were obtained from dichloromethane/cyclopentane by diffusion [30]. Data were collected at 183(2) K with Mo K_α radiation (λ = 0.7107 Å) that was graphite-monochromated on an Oxford Diffraction CCD Xcalibur system with a Ruby detector. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), placed on a nylon loop that is mounted in a CrystalCap Magnetic™ (Hampton Research) and immediately transferred to the diffractometer. The program suite $CrysAlis^{Pro}$ was used for data collection, multi-scan absorption correction and data reduction [31]. The structure was solved with direct methods using SIR97 [32] and was refined by full-matrix least-squares methods on F^2 with SHELXL-97 [33]. CCDC 928749 contains the crystallographic data for this compound. Molecular formula: C_{17} H_{16} O_5; M: 300.3; Crystal system: Monoclinic; Space group: C2/c; a = 20.8030(13) Å; b = 7.4897(5) Å; c = 18.5544(12) Å;β = 90.835(6) °; Z = 8; D_{calc} = 1.380 Mg/m^3; V = 2890.6(3) $Å^3$; μ = 0.102 mm^{-1}; Crystal size: 0.40 × 0.35 × 0.09 (mm); θ range for data collection: 2.92 - 30.51; Index ranges: $-29 \le h \le 28$, $-10 \le k \le 9$, $-26 \le l \le 26$; Max. and min. transmission: 0.9909 and 0.7028; F(000): 1264; Data/restraints/parameters: 4053/0/200; Goodness-of-fit on F^2: 1.058; Reflections collected: 9493; Independent reflections: 4053 [R(int) = 0.0298]; Final R indices [I >2sigma(I)]: R1 = 0.0590, wR2 = 0.1537; R indices (all data): R1 = 0.0768, wR2 = 0.1682.

Synthesis of 4

To a stirring suspension of NaH (0.14 g, 5.8 mmol) in dry THF (30 mL) under a nitrogen atmosphere, a solution of **3** (0.20 g, 0.3 mmol) in dry THF (10 mL) was added at room temperature. 1,4–Bis(bromomethyl) benzene (0.039 g, 0.15 mmol) was dissolved in dry THF (10 mL) and added dropwise to the above solution during 10 min. The temperature was increased and the reaction was refluxed over night. The solvent was removed under reduced pressure with rotary evaporator and the solid was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL). The organic phase was separated and dried with magnesium sulfate and the solvent was evaporated under reduced pressure with rotary evaporator. The oily compound was purified by chromatography on aluminum oxide neutral. Elution was started with hexane. The polarity of eluent was increased gradually to 1:2 EtOAc-hexane. The product was obtained as a light yellow solid; yield: 0.12 g (56 % based on **3**). R_f = 0.5 (1:2 EtOAc-hexane).

FT-IR (KBr): 3038 m ($CH_{aromatic}$), 2979 m ($CH_{aliphatic}$), 1694 s (C=O), 1600 m, 1456 s 1414 s ($C=C_{aromatic}$), 1243 s ($C\text{-}O\text{-}C_{asym}$), 1162 s ($C\text{-}O\text{-}C_{sym}$), 1041 w (C-N), 755 s ($=C\text{-}H_{aromatic})_{o.o.p}$ cm^{-1}.

Anal. Calcd. for $C_{80}H_{112}N_6O_{18}$: C, 66.46; H, 7.81; N, 5.81 %. Found: C, 66.44; H, 7.80; N, 5.76 %. MS (ESI): m/z (%) = 1468 (100 $[M + Na]^+$). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.38 (s, 36H, $C(C\mathbf{H}_3)_3$), 1.42 (s, 18H, $C(C\mathbf{H}_3)_3$), 2.93 - 3.31 (br m, 16H, $NC\mathbf{H}_2C\mathbf{H}_2N$), 4.18 - 4.22 (br m, 10H, $ArC\mathbf{H}_2N$, C**H**O) , 4.44 - 4.52 (br m, 8H, $ArOC\mathbf{H}_2$) 4.74 (s, 4H, $ArC\mathbf{H}_2$ core), 6.87 (d , 4H, J = 8 Hz, Ar**H**), 6.93 (t, 4H, J = 8 Hz, Ar**H**), 7.18 - 7.22 (m, 8H, Ar**H**), 7.33 (s, 4H, Ar**H** core) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 28.68, 29.49, $(C(\mathbf{C}H_3)_3)$, 46.42 ($N\mathbf{C}H_2\mathbf{C}H_2N$), 67.76, 68.09 ($Ar\mathbf{C}H_2N$, $ArO\mathbf{C}H_2$), 72.50 ($Ar\mathbf{C}H_2$ core), 79.69, 79.99 ($\mathbf{C}(CH_3)_3$), 112.18 (Ar), 121.68 (Ar), 128.47 (Ar), 128.80 (Ar), 137.86 (Ar), 155.38 (Ar), 156.02, 156.63 (**C**O) ppm.

Synthesis of 5

4 (0.5 g, 0.35 mmol) was added to a stirred mixture of dichloromethane (20 mL) and trifluoroacetic acid (20 mL). The mixture was stirred at room temperature for 2 h. After removal of the solvent by rotary evaporator, methanol was added and then evaporated again to dryness. The mixture was partitioned between aqueous sodium carbonate (15 %, 50 mL) and dichloromethane (50 mL). The combined organic phases were dried over magnesium sulfate, and the solvent removed to yield **5** as creamy precipitate solid (0.2 g, 70 %). Mp.: 86-89 ˚C, R_f = 0.25 (1:20 conc. NH_3-MeOH).

Anal. Calcd. for $C_{50}H_{64}N_6O_6.H_2O.CH_2Cl_2$: C, 64.61; H, 7.23; N, 8.86 %. Found: C, 64.50; H, 7.34; N, 8.54 %. MS (ESI): m/z (%) = 845 (100 $[M + H]^+$), 423 (4 $[M + 2H]^{2+}$). FT-IR (KBr): 3350 w (NH), 2916 m ($CH_{aliphatic}$), 1600 m, 1491 s, 1450 s ($C=C_{aromatic}$), 1236 s (C-O-C_{asym}), 1111 s (C-O-C_{sym}), 1047 s (C-N), 751 s (=C-$H_{aromatic}$)$_{o.o.p}$ cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.47 - 2.55 (m, 16H, NC**H**$_2$C**H**$_2$N), 3.71 - 3.80 (dd, 8H, J = 12, 8 Hz, ArC**H**$_2$), 4.18 - 4.28 (m, 10H, ArOC**H**$_2$, C**H**O), 4.72 (s, 4H, ArC**H**$_2$ core), 6.87 - 6.91 (m, 8H, Ar**H**), 7.15 (d, 4H, J = 8 Hz, Ar**H**), 7.21 (t, 4H, J = 8 Hz, Ar**H**), 7.30 (s, 4H, Ar**H** core) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 49.13, 49.23 (NH**C**H$_2$**C**H$_2$NH), 50.88 (Ar**C**H$_2$N), 67.79 (ArO**C**H$_2$), 72.50 (Ar**C**H$_2$ core), 76.77 (**C**HO), 112.10 (Ar), 121.23 (Ar), 128.40 (Ar), 128.49 (Ar), 129.16 (Ar), 131.25 (Ar core), 137.38 (Ar core), 157.34 (Ar) ppm. $^{13}C\{DEPT, 135\}$ (100 MHz, $CDCl_3$): aliphatic δ = CH_2; 49.72, 49.83, 51.47, 68.37, 73.08, aliphatic CH; 77.35, aromatic CH; 112.68, 121.86, 129.04, 129.73, 131.83 ppm.

Synthesis of 6

To the stirring suspension of NaH (0.20 g, 8.3 mmol) in dry THF (30 mL) under nitrogen atmosphere, solution of **3** (0.5 g, 0.75 mmol) in dry THF (20 mL) was added at room temperature. Benzylbromide (0.09 mL, 0.75 mmol) was dissolved in dry THF (10 mL) and added dropwise to the above solution during 10 min. The temperature was increased and the reaction was refluxed over night. The solvent was removed under reduced pressure with rotary evaporator and the solid was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL). The organic phase was separated and dried with magnesium sulfate and the solvent was evaporated under reduced pressure by using a rotary evaporator. The oily compound was purified by chromatography on silica gel. Elution was started with hexane. The polarity of eluent was increased gradually to 2:5 EtOAc-hexane. The product was obtained as a light yellow solid; Yield: 0.43 g (76 % based on **3**). R_f = 0.7 (2:5 EtOAc-hexane).

Anal. Calcd. for $C_{43}H_{59}N_3O_9$: C, 67.78; H, 7.80; N, 5.51 %. Found: C, 67.77; H, 7.66; N, 5.40 %. MS (ESI): m/z (%) = 800 (50 $[M + K]^+$), 784 (100 $[M + Na]^+$), 762 (42 $[M + H]^+$), 662 (5 $[M\text{-Boc} + 2H]^+$). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.39, 1.42 (two distinct singlets, 27H, C(C**H**$_3$)$_3$), 3.02 - 3.30 (br m, 8H, NC**H**$_2$C**H**$_2$N), 4.18 - 4.24 (m, 5H, ArOC**H**$_2$, C**H**O) 4.43 (br s, 4H, ArC**H**$_2$N), 4.74 (s, 2H, ArC**H**$_2$O), 6.87 (d, 2H, J = 8 Hz, Ar**H**), 6.94 (t, 2H, J = 8 Hz, Ar**H**), 7.13 - 7.22 (m , 4H, Ar**H**), 7.28 - 7.30 (m, 1H, Ar**H** link), 7.33 - 7.34 (m, 4H, Ar**H** link) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 28.68, 29.27 (C(**C**H$_3$)$_3$), 45.34, 46.41 (N**C**H$_2$**C**H$_2$N), 68.04 (ArO**C**H$_2$), 68.82 (Ar**C**H$_2$N), 72.79 (Ar**C**H$_2$), 76.73 (**C**HO), 79.69, 79.98 (**C**(CH$_3$)$_3$), 112.18 (Ar), 121.67 (Ar), 126.83 (Ar link),

127.20 (Ar link), 128.08 (Ar), 128.18 (Ar), 128.75 (Ar link), 133.18 (Ar), 137.87 (Ar link), 155.38, 156.02 (**C**O), 156.65 (Ar) ppm.

Synthesis of 7

6 (0.50 g, 0.65 mmol) was added to a stirred mixture of dichloromethane (15 mL) and trifluoroacetic acid (15 mL). The mixture was stirred at room temperature for 2 h. After removal of the solvent by the rotary evaporator, methanol was added and then evaporated again to dryness. The mixture was partitioned between aqueous sodium carbonate (15 %, 50 mL) and dichloromethane (50 mL). The organic phase was dried over magnesium sulfate, and the solvent removed to yield **7** as light brown precipitate solid (0.22 g, 64 %). R_f = 0.5 (1:20 conc. NH_3-MeOH).

Anal. Calcd. for $C_{28}H_{35}N_3O_3.0.5CH_2Cl_2$: C, 67.91; H, 7.20; N, 8.34 %. Found: C, 67.48; H, 6.89; N, 8.63 %. FT-IR (KBr): 3500 w (NH), 2937 m ($CH_{aliphatic}$), 1600 m, 1494 s, 1454 s ($C=C_{aromatic}$), 1247 s ($C\text{-}O\text{-}C_{asym}$), 1121 m ($C\text{-}O\text{-}C_{sym}$), 1050 m (C-N), 753 s ($=C\text{-}H_{aromatic})_{o.o.p}$ cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 2.44, 2.51 (two distinct singlets, 8H, NHC**H**$_2$C**H**$_2$NH), 3.06 (br, s, 3H, N**H**), 3.68 - 3.75 (dd, 4H, J = 15, 19 Hz, ArC**H**$_2$N), 4.05 - 4.22 (m, 5H, ArOC**H**$_2$, C**H**O), 4.65 (s, 2H, ArC**H**$_2$ link), 6.81 - 6.85 (m, 4H, Ar**H**), 7.10 (d, 2H, J = 5 Hz, Ar**H**), 7.15 (t, 2H, J = 10 Hz, Ar**H**), 7.22 - 7.25 (m, 5H, Ar**H** link) ppm. $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ = 48.63, 48.70 (NH**C**H$_2$**C**H$_2$NH), 50.41 (Ar**C**H$_2$N), 67.48 (ArO**C**H$_2$), 72.44 (Ar**C**H$_2$ link), 76.38 (**C**HO), 111.78 (Ar), 121.04 (Ar), 128.00 (Ar), 128.11 (Ar), 128.53 (Ar), 128.91 (Ar link), 130.91 (Ar link), 137.80 (Ar link), 156.99 (Ar) ppm. MS (EI): m/z (%) = 461 (11 $[M]^+$), 91 (100 $[C_7H_7]^+$).

Metal complexes synthesis

Synthesis of $Cu(5)(NO_3)_2.3H_2O$ (8)

A methanol solution (10 mL) of **5** (0.20 g, 0.23 mmol) was added slowly to a 10 mL hot methanol solution of $Cu(NO_3)_2.3H_2O$ (0.11 g, 0.46 mmol) with stirring during 5 min. The solution was stirred and refluxed for 2 h. The solvent was removed by using a rotary evaporator. The resultant solid products were washed by diethylether and acetonitrile and collected by filtration. After recrystalization from acetonitrile and tetrahydropyrane [29] blue precipitates was obtained. Yield: 0.13 g (52 % based on **5**).

Anal. Calcd. for $C_{50}H_{64}CuN_{12}O_8.3H_2O$: C, 55.26; H, 6.49; N, 10.31 %. Found: C, 55.13; H, 6.68; N, 9.95 %. FT-IR (KBr): 3442 s (OH), 2929 s ($CH_{aliphatic}$), 1602 s, 1493 s, 1454 s ($C=C_{aromatic}$), 1384 s (NO_3^-), 1238 s ($C\text{-}O\text{-}C_{asym}$), 1115

s (C-O-C_{sym}), 758 s (=C-$H_{aromatic}$)$_{o.o.p}$ cm^{-1}. MS (MALDI): m/z (%) = 923 (3 [**5**+Cu+O]$^+$), 907 (14 [**5**+Cu]$^+$), 845 (100 [**5**]$^+$), 841 (94 [**5**-4H] $^+$).

Synthesis of Cu(7)$(NO_3)_2$.2.5H_2O complex (9)

A methanol solution (10 mL) of **7** (0.20 g, 0.43 mmol) was added slowly to a 10 mL hot methanol solution of $Cu(NO_3)_2$.3H_2O (0.10 g, 0.43 mmol) with stirring during 5 min. The solution was stirred and refluxed for 2 h. The solvent was removed by using a rotary evaporator. The resultant solid products were washed by diethylether and acetone and collected by filtration. After recrystalization form methanol and n-hexane, blue precipitates were obtained. Yield: 0.2 g (66 % based on **7**).

Anal. Calcd. for $C_{28}H_{35}CuN_5O_9$.2.5H_2O: C, 48.45; H, 5.81; N, 10.09 %. Found: C, 48.57; H, 6.04; N, 9.76 %. FT-IR (KBr): 3198 br w (OH, NH), 2925 s ($CH_{aliphatic}$), 1632 s (δ N-H), 1565 s, 1494 s, 1444 s (C=$C_{aromatic}$), 1367 s (NO_3^-), 1241 s (C-O-C_{asym}), 1110 s (C-O-C_{sym}), 760 s (=C-$H_{aromatic}$)$_{o.o.p}$ cm^{-1}.

Results and discussion

Syntheses and characterization

The synthesis route for obtaining the target compounds is depicted in Scheme 1. The X-ray structural analysis of the 2-[3-(2-formyl phenoxy)-2-hydroxyl propoxy] benzaldehyde **1** shows that the dialdehyde adopts a conformation in which both etheric oxygens connected to both side of the 2-propanol and both of the aldehyde groups are adopting anti arrangement (**Fig. 1**).

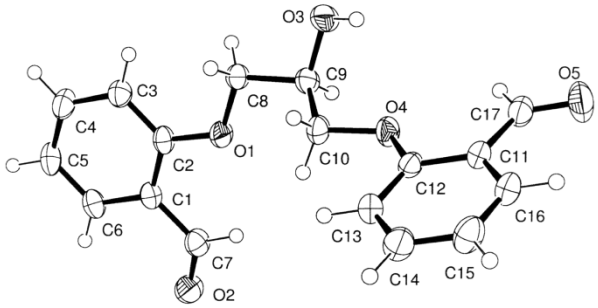
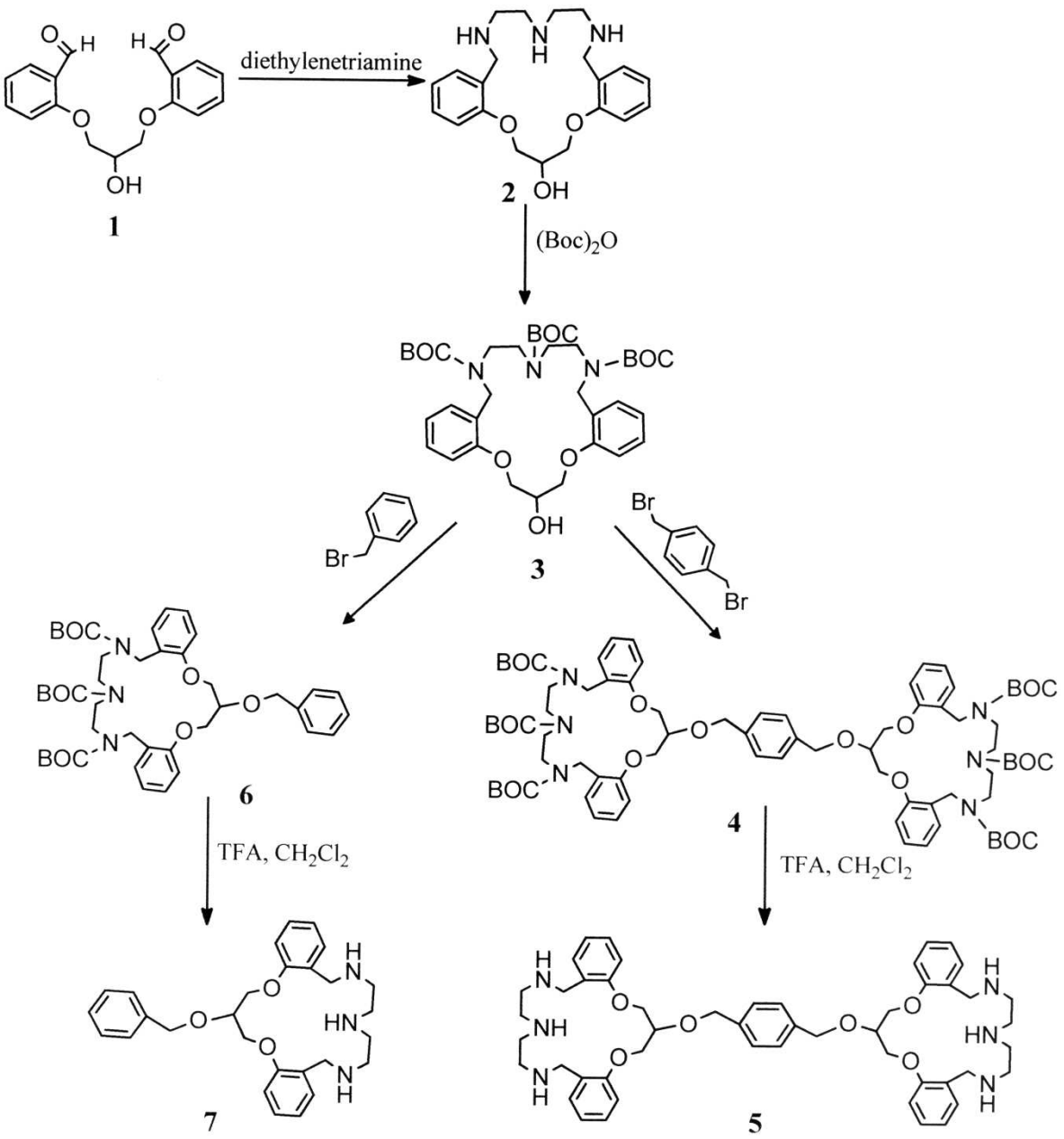


Fig. 1: ORTEP representation of **1** shown with 50% probability

The starting macrocycle **2** was produced by the reaction of **1** and diethylenetriamine according to the published procedure [28]. The reaction of **2** with di-tert-butyl dicarbonate $(Boc)_2O$ in methanol produced the N-protected

189 macrocycle **3**. Synthesis of target compounds **5** and **7** started by alkylation of **3** as shown in Scheme 1. **3** was
190 deprotonated with NaH as a base in dry THF and the resulting deprotonated **3** treated with bromomethyl benzene
191 derivatives. The isolation of the products required purification by chromatography on either silica gel or neutral
192 aluminum oxide. Compound **4** was obtained by alkylation of **3** with 1,4-dibromomethyl benzene in 56 % yield.
193 Characterization of the **4** by ESI-MS showed the $[M + Na]^+$ molecular ion at m/z = 1468 (**Fig. S1**). In order to
194 synthesize **6**, **3** was treated with bromomethyl benzene and **6** was obtained in 76 % yield. In the ESI-MS of **6** the
195 expected peak for the $[M + Na^+]$ molecular ion appeared at m/z = 784. Interpretation of the ^1H NMR spectra of the
196 Boc derivatives was complicated by signal broadening due to the slow rotation around the amide bonds. This is no
197 longer the case for the final products after deprotection. **5** and **7** with two and one macrocyclic cavities respectively
198 were obtained by deprotection of **4** and **6** by using trifluoroacetic acid as shown in Scheme 1. In the ^1H NMR of **5**
199 additional peaks at 4.72 ppm for CH_2 aliphatic and at 7.30 ppm for aromatic protons of linker were observed (**Fig.**
200 **S2**). In $^{13}C\{^1H\}$ NMR, signal for CH_2 of linker appears at 72.50 ppm (**Fig. S3**). In addition, ^{13}C DEPT NMR for **5**
201 clearly shows all aliphatic and aromatic -CH_2-, and -CH- groups (**Fig. S4**). The ESI-MS of **5** showed the expected
202 peak for the $[M + H]^+$ molecular ion at m/z = 845 (**Fig S5**). ^1H and $^{13}C\{^1H\}$ NMR confirmed structure of **7** (**Fig. S6**
203 and **Fig. S7**). The EI-MS spectrum provided evidence for the structure of **7** with the expected peak of $[M + H]^+$ at
204 m/z = 461.
205 Reaction of the ligands with $Cu(NO_3)_2.3H_2O$ gave mononuclear complexes of copper(II). Elemental analyses for the
206 complexes fit well with 1:1 metal to ligand ratio. Surprisingly, also the potentially dinucleating ligand **5** reacted with
207 copper(II) nitrate to give a mononuclear complex. The molecular weight of bis-macrocycle complex **8** was
208 established from the molecular ion peaks observed in the corresponding MALDI mass spectrum (**Fig. S8**).



Scheme 1: Synthetic routes to obtain intermediates **4**, **6** and **5** and **7** as products

Complexation of 5 and 7 with Cu(II) in solution

Stoichiometry of complexes formed by **5** and **7** with Cu(II) in methanol solution were determined under the same condition. The reaction equilibria involving macrocyclic ligands and copper(II) nitrate in methanol have been followed spectrophotometrically by observing the spectral changes that occur on the incremental addition of the metal ion to the ligand solution till no further change is seen. Ligands **5** and **7** show no absorption band in 400-800

nm region. Upon addition of a Cu(II) ion solution to solution of ligand a new broad band appears in the spectrum that is different to the spectra for the free ligand and free metal salt (**Fig. 2**). The variation of absorption at 645 nm and 647 nm with the number of equivalents of Cu(II) are shown in (**Fig. 2**) for **5** and **7,** respectively. The observed data indicate clear formation of 1:1 metal to ligand species for both compounds. Therefore **5** having two macrocyclic rings incorporates only one copper cation. The results are consistent with elemental analysis of compounds in solid state and MALDI mass.

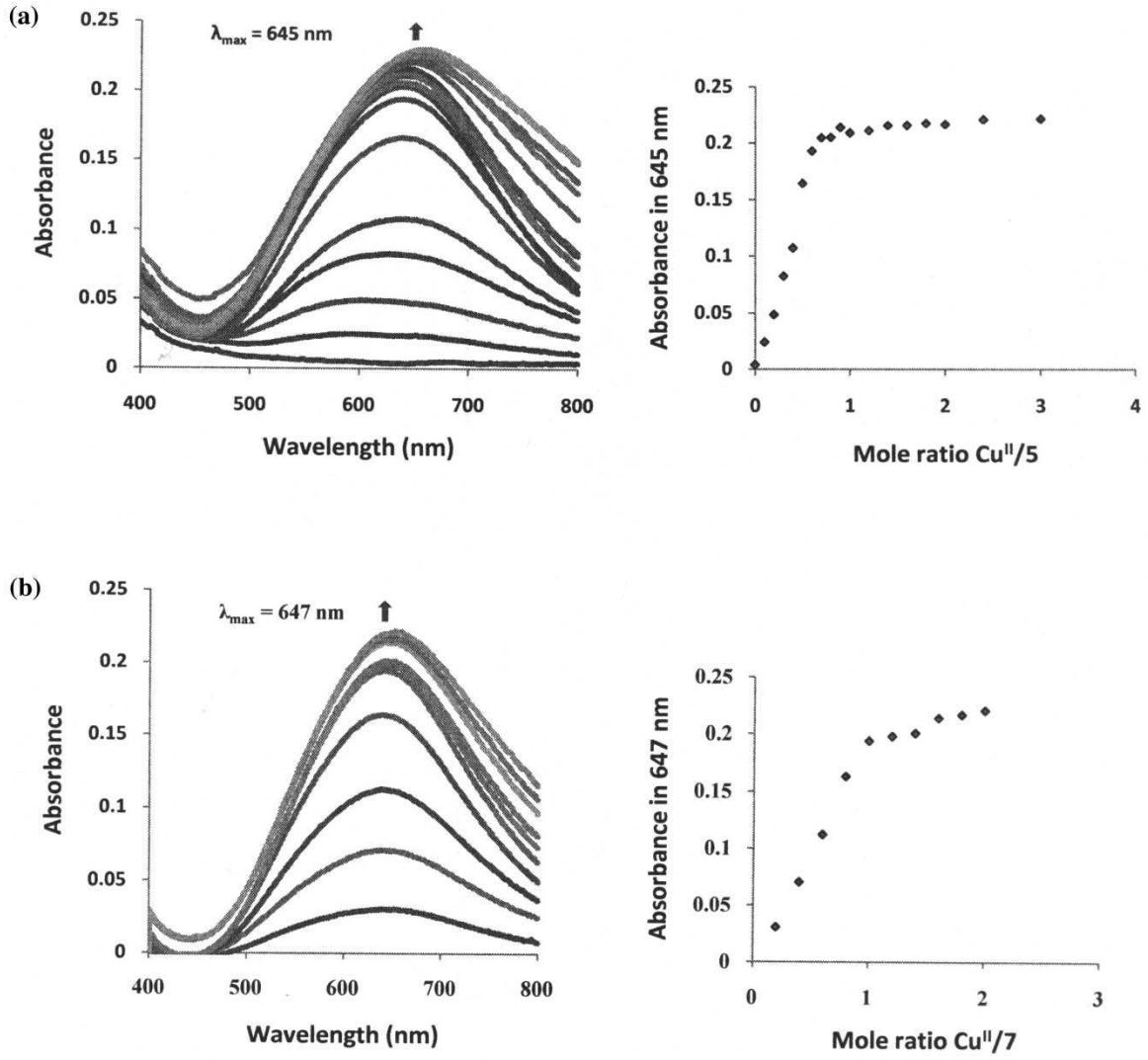


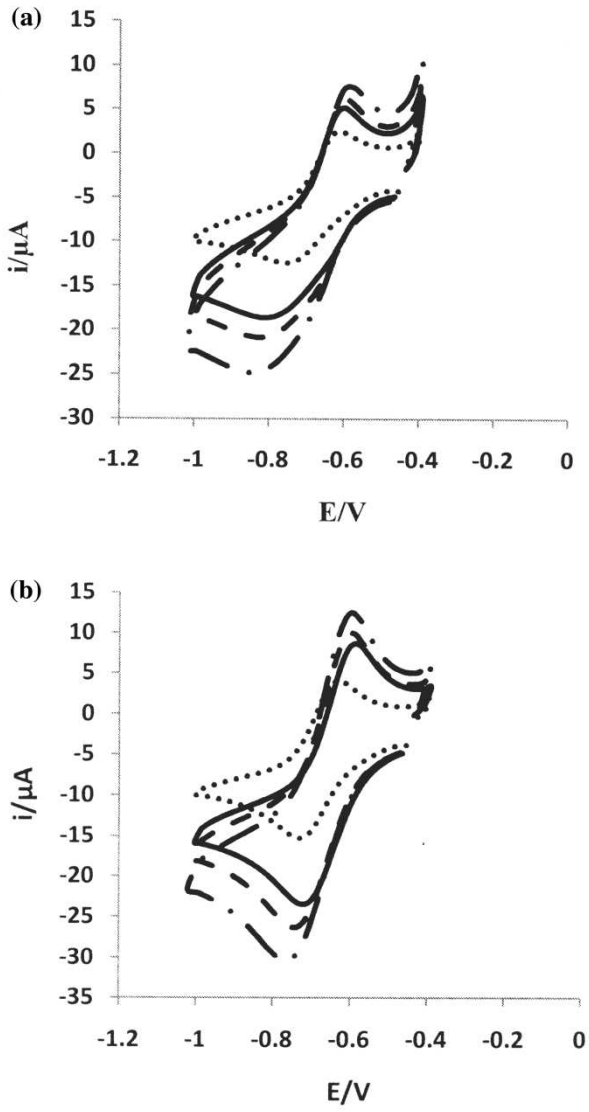
Fig. 2: (a) UV-Vis titration of 5 (1.25×10^{-3} M) with Cu(II) nitrate in CH_3OH, (b) UV-Vis titration of 7 (1.25×10^{-3} M) with Cu(II) nitrate in CH_3OH

Cyclic voltammetry

The electrochemical properties of the metal complexes **8** and **9** were investigated in DMF containing 0.05 M KNO_3 as supporting electrolyte. The obtained data in this work are listed in Table 1. **Fig. 3** represents the cyclic voltammograms of complexes **8** and **9** in different scan rates. The voltammograms display one cathodic peak of the reduction process Cu^{II}/Cu^{I} in 50 mVs^{-1} scan rate at E_c = -752 and -718 mV and their corresponding anodic peaks at E_a = -611 and -644 mV for **8** and **9**, respectively. The peak separation is 141 mV for **8** and 74 mV for **9** indicate quasi-reversible and reversible process for **8** and **9**, respectively, in comparison to peak separation of Fc/Fc^{+} (ΔE = 90 mV) in the same condition. These complexes could be good candidates to use as electroactive catalyst in oxidation- reduction reactions.

Table 1. Cyclic voltammetric data for complexes (scan rates from left to right are 50, 150, 200 and 300 mVs^{-1}) **8** and **9**.

compound	E_c (mV)	E_a (mV)
8	-752, -823, -829, -840	-611, -599, -589, -570
9	-718, -719, -749, -750	-644, -584, -609, -600
ferrocene	460	550



240

241 **Fig. 3.** Cyclic voltammograms of complex **8** (a) and complex **9** (b) in DMF solution containing 0.05 M KNO_3 in
242 different scan rates. 50 mVs^{-1} (dotted line), 150 mVs^{-1} (solid line), 200 mVs^{-1} (dashed line), 300 mVs^{-1} (dashed-
243 dotted line)

244

245 **Cleavage of plasmid DNA:** pGS2 DNA (650 ng/μL) in cacodylate buffer (0.1 mM, pH 7.5) containing (50 mM
246 NaCl) was treated with copper complex **8** and 1 μL of 3-mercaptopropionic acid (MPA), 20 mM to yield a total
247 volume of 10 μL. The mixture was then incubated for 16 h at 37 ˚C. The reaction was quenched by the addition of 2
248 μL blue dye (50 mM) and then the resulting solutions were loaded on 1 % agarose gel. Electrophoresis was carried
249 out at 70 mV for 3 h in a TAE buffer (40 mM Tris acetate/1 mM EDTA pH 8). Bands were visualized under

transilluminator light and photographed. DNA plasmid was incubated with different concentrations of the metal complex. The results after gel electrophoresis are shown in **Fig. 4**.

According to **Fig 4**, this complex with two macrocycle rings is able to cause degradation of DNA. The activity of this complex is increased with increasing concentration of complex. In the lanes 2, 3, 4, 5 the supercoiled DNA plasmid (Form I) were converted to nicked (form II) and linear (Form III), whereas all of the supercoiled form (Form I) converted to linear form (Form III) completely in the lane 6 at 0.5 mM concentration of complex. While the different concentrations of copper nitrate have been able to degrade DNA to small species (**Fig. S9**) the bis-macrocycle copper(II) complex has nevertheless a good activity in degrading supercoiled form to the nicked and linear forms.

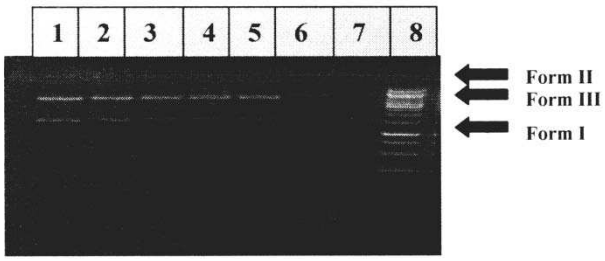


Fig 4: Agarose gel electrophoresis patterns for the cleavage of pGS2 plasmid DNA (650 ng/μL) by complex **8** in the presence of 200-fold excess of MPA in the dark for 16 h in cacodylate buffer (0.1 mM, pH 7.5, containing 50 mM NaCl) 37 ˚C: Lane 1, DNA + MPA; lane 2, DNA + MPA + **8** (0.05 mM); lane 3, DNA + MPA + **8** (0.1 mM); lane 4, DNA + MPA + **8** (0.15 mM); lane 5, DNA + MPA + **8** (0.25 mM); lane 6, DNA + MPA + **8** (0.5 mM); lane 7, DNA + MPA + **8** (1 mM); lane 8: marker, Gene Ruler, 1 kb DNA, Fermentas.

Conclusion

In conclusion, we have presented the successful and clean synthesis of the new bis-macrocycle **5** and mono-cyclic analogue **7** by using simple protecting group strategy and Williamson etherification chemistry. Complexes **8** and **9** were synthesized and formulated by elemental analysis as 1:1 metal to ligand complexes. Also UV-Vis titration of **5** and **7** with copper(II) nitrate indicated the 1:1 metal: ligand complex formation in alcoholic solution. **8** and **9** show, respectively, quasi-reversible and reversible Cu^{II}/Cu^{I} redox couple. Complex **8** cleaved plasmid pGS2 DNA by using an oxidative mechanism with 3- mercaptopropionic acid (MPA) as the reductant under aerobic conditions.

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Supplementary data

Supplementary data associated ^{1}H, ^{13}C NMR of **5**, **7**, DEPT ^{13}C NMR of **5**, ESI-MS of **4**, **5**, MALDI–MS for **8**, DNA cleavage by $Cu(NO_3)_2.3H_2O$

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